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MEDICAL AND DENTAL EDUCATION

Anatomy-Based DMEK Wetlab in Homburg/Saar:
Novel Aspects of Donor Preparation and Host Maneuvers to Teach Descemet Membrane Endothelial Keratoplasty

B. SEITZ ,* L. DAAS, M. BISCHOFF-JUNG, N. SZENTMÁRY, S. SUFFO, M. EL-HUSSEINY, A. VIESTENZ, AND G. MILIOTI

Department of Ophthalmology, Saarland University Medical Center UKS, Homburg /Saar, Saarland, Germany

Use of Descemet Membrane Endothelial Keratoplasty (DMEK) has been limited because of problems with donor preparation, i.e. tearing of the Descemet membrane and difficulties in unfolding the Endothelium-Descemet-Membrane-Layer (EDML) in the anterior chamber (AC). The purpose of this work was to describe a novel approach to teaching anatomy-based donor and recipient preparation in a DMEK-Wetlab. We teach successful mono-manual donor preparation of human corneas in organ culture not suitable for transplantation, including peripheral markers for orientation. We also teach safe recipient preparation in a freshly-enucleated pig eye in organ culture preservation medium for atraumatic introduction of the EDML roll into the AC, reliable orientation of the EDML during surgery, and stepwise unfolding within the AC. Twenty-two candidates in the 1. Homburg Cornea Curriculum HCC 2015 who practiced both preparations using three human donor corneas and three pig eyes assessed the procedure as follows: (1) overall grade of the Wetlab 1.4 (median 1, range 1 to 2 - on a scale from 1 (excellent) to 6 (terrible); (2) most participants and tutors stated that the Wetlab is most effective for colleagues who have some previous experience with corneal microsurgery. Our novel anatomy-based approach to simulating donor preparation and graft implantation for DMEK seems to meet the expectations and requirements of colleagues with previous experience in corneal microsurgery and will help to reduce the rate of complications for incipient DMEK surgeons in the future. Clin. Anat. 31:16–27, 2018.

Key words: descemet membrane endothelial keratoplasty; DMEK; lamellar keratoplasty; Wetlab; donor preparation; recipient/host maneuvers; corneal endothelial dystrophy

INTRODUCTION

During recent years it has been claimed in ophthalmic congress lecture halls that on the basis of the pathology 50% of all cornea transplantations could be operated as posterior lamellar keratoplasty. The "German Keratoplasty Registry", which was established by the Cornea Section of the German Ophthalmological Society (Deutsche Ophthalmologische Gesellschaft DOG) in 2000, showed in 2015 a 43.7% proportion for penetrating keratoplasty (PKP), a 53.2% proportion for posterior lamellar keratoplasty [mainly DMEK, Descemet Membrane Endothelial Keratoplasty ($n = 3326$) versus DSAEK, Descemet Stripping Automated Endothelial Keratoplasty ($n = 330$)], but only 3.1% for anterior lamellar keratoplasty [mainly DALK, Deep Anterior Lamellar

*Correspondence to: Prof. Dr. med. Berthold Seitz ML, FEBO, Department of Ophthalmology, Saarland University Medical Center UKS, D-66424 Homburg/Saar, Saarland, Germany.
E-mail: berthold.seitz@uks.eu

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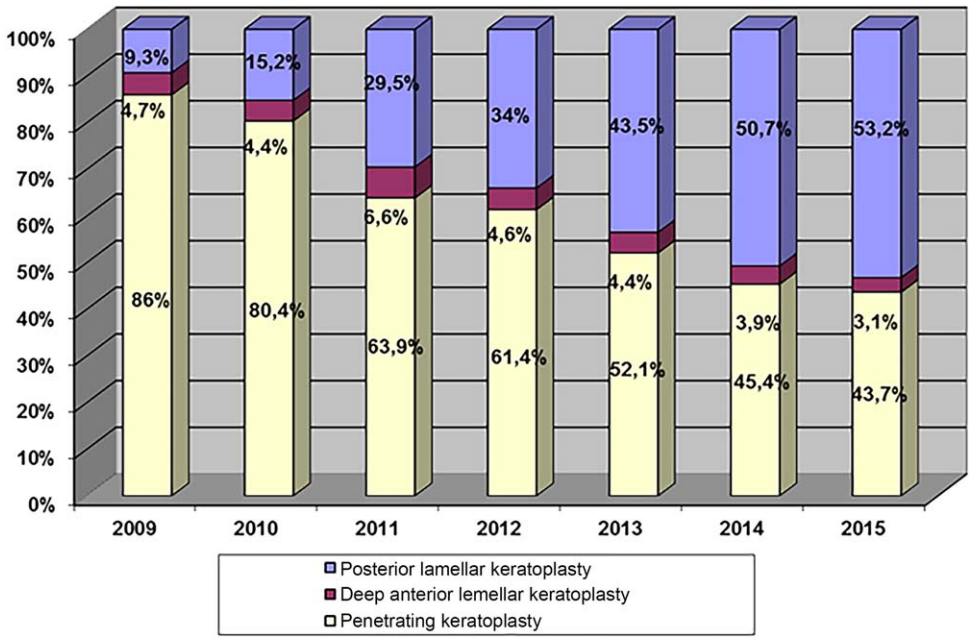

Fig. 1. German Keratoplasty Registry by the Cornea Section of the DOG. In 2015, 43.7% of all grafts were penetrating (PKP), 53.2% posterior lamellar (DMEK or DSAEK), and only 3.1% anterior lamellar keratoplasties (DALK). [Color figure can be viewed at wileyonlinelibrary.com]

Keratoplasty ($n = 214$)] (Fig. 1). In 2015, the number of keratoplasties in Germany increased to almost 7000 (Fig. 2). Over the last 11 years, the number of keratoplasties in Homburg/Saar has increased about sevenfold (Fig. 3). The number of processed donor tissues in our LIONS Eye Bank Saar-Lor-Lux, Trier/Westpfalz has almost tripled since its foundation in 2000 (Fig. 4).

Indications for Keratoplasty

Apart from methodological problems with lamellar techniques, there are several indications that still in principle need a **PKP** (Seitz, 2016). These indications can be divided into elective (optic versus tectonic) and curative emergency (PKP à chaud) operations. A peripheral/eccentric tectonic corneoscleroplasty is needed in the event of a block excision, for example due to a malignant melanoma of the ciliary body or an epithelial implantation cyst, "traumatic iridenkleisis", or half-moon-shaped rheumatoid corneal melting at the limbus. The central round 14 mm tectonic corneoscleroplasty can be useful in keratoglobus or advanced keratotorus (= pellucid marginal degeneration). An infectious or rheumatic descemetocele or corneal perforation makes a PKP à chaud necessary for restoring

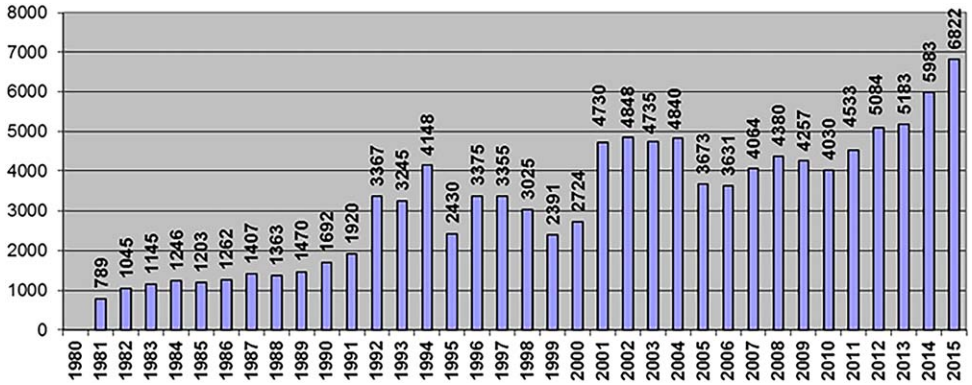

Fig. 2. German Keratoplasty Registry by the Cornea Section of the DOG. In 2015, 6,822 keratoplasties were performed overall. At the end of 2015, 3,582 were on the decentral waiting lists of German Eye Banks. [Color figure can be viewed at wileyonlinelibrary.com]

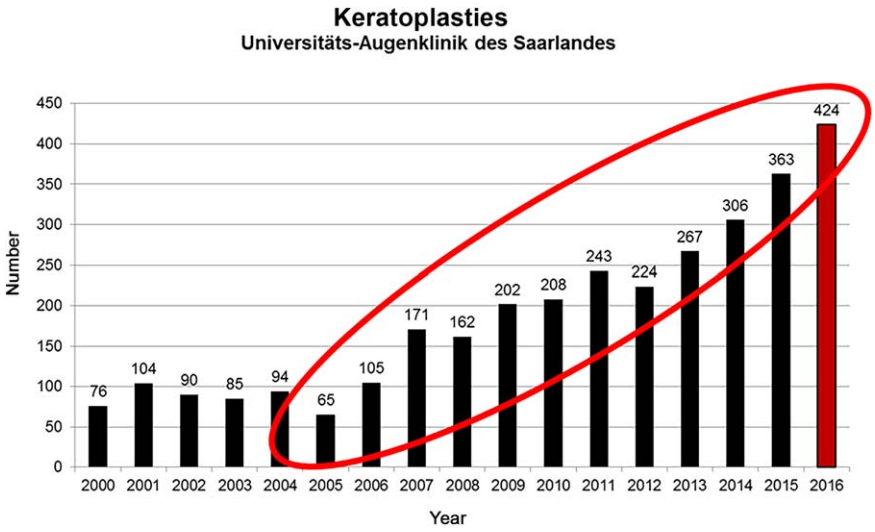

Fig. 3. Over the last 11 years, the number of keratoplasties in Homburg/Saar has increased about sevenfold. [Color figure can be viewed at wileyonlinelibrary.com]

the integrity of the globe. Classically, an elective optic central PKP is necessary in through-and-through corneal pathologies, for example, macular corneal dystrophy, repeat keratoplasty in the event of high astigmatism, or keratoconus "recurrence" because of too small a previous graft size. In small decentered grafts with high irregular astigmatism, a well-centered repeat keratoplasty with larger diameter [preferably excimer laser trephination (Seitz, 1999, 2016) with maximum diameter of 8.5 mm] is often the only useful option. Scars after acute keratoconus with a defect in Descemet's membrane (DM) and corneal hydrops, central scars after penetrating corneal injury, and long-standing endothelial-epithelial decompensation with serious (partly vascularized) stromal scars, also need PKP. Excimer laser PKP in aphakic eyes requires a "Flieringa ring" to prevent collapse of the globe in the open-eye situation, especially if the eye had already undergone vitrectomy. The herpes simplex virus can persist in the endothelium. In these cases, to avoid viral recurrence, neither DALK nor ipsilateral autologous rotational keratoplasty should be performed. In acanthamebal or fungal keratitis (especially fusaria) very large transplant diameters are necessary to remove the pathogens in toto as quickly as possible (Seitz, 2013). Nowadays, donor tissue for PKP should be routinely investigated in the Eye Bank by "donor tomography" to avoid post-refractive surgery and keratoconus-caused surface irregularities (Damian, 2017).

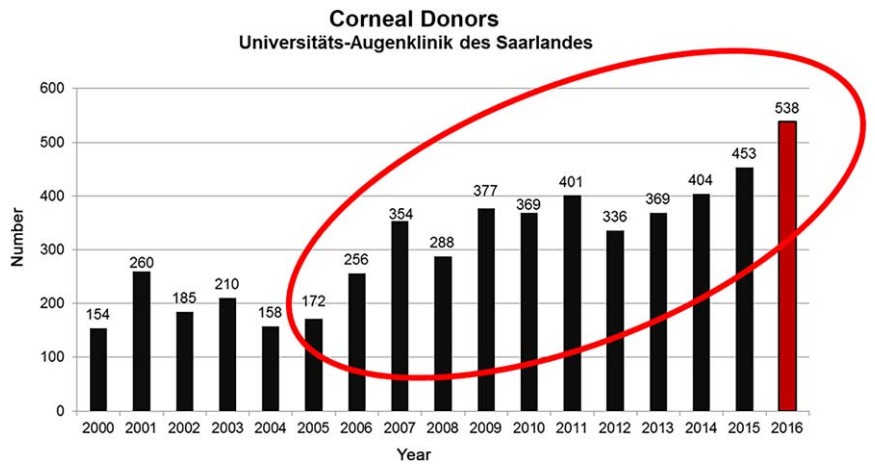

Fig. 4. The number of processed donor tissue in our LIONS Eye Bank Saar-Lor-Lux, Trier/Westpfalz, has almost tripled since its foundation in 2000—not least pushed by the effective cooperation with hospitals in Luxembourg. [Color figure can be viewed at wileyonlinelibrary.com]

Indications for **DALK** include in principle corneal pathologies with intact endothelium and intact DM (Seitz, 2013): keratoconus, stromal dystrophies without endothelial involvement (e.g., Schnyder corneal dystrophy), and epithelial-stromal TGFBI dystrophies (e.g., granular and lattice dystrophies), especially after unsuccessful phototherapeutic keratectomy (Seitz, 2015).

Indications for **posterior lamellar keratoplasty** include in principle all diseases of the endothelium with stromal edema but without permanent stromal scars [e.g., Fuchs endothelial corneal dystrophy (Seitz and Hager, 2017), pseudophakic bullous keratopathy, and endothelial degeneration of viral genesis].

Since its first description by Gerit Melles in 2006, DMEK has enjoyed a well-deserved rapid boost in Germany over the past 10 years. The following well-known potential postoperative problems of PKP have been minimized by the application of DMEK: (1) high/irregular astigmatism, (2) surface problems, and (3) biomechanical instability. The major advantages of DMEK over PKP are: (1) faster visual recovery in a few days or weeks postoperatively, (2) better final visual acuity: 60% > 0.8 after one year, (3) less immune reaction: 15× less than after PKP (Anshu et al., 2012; Szentmáry et al., 2013), (4) no suture-associated problems, and (5) no expulsive hemorrhage (Seitz et al. 2013).

Although experience with DMEK yields outcomes of unsurpassed quality, its wider use has been limited because of technical problems with donor preparation, that is, tearing of the Descemet membrane and difficulties in unfolding the Endothelium-Descemet-Membrane-Layer (EDML) in the anterior chamber (AC). Although the initial descriptions of DMEK suggested that donor tissue preparation and manipulation in the AC could be performed routinely, tearing of the EDML results in consecutive graft loss, and prolonged manipulation of EDML in the AC results in significant endothelial loss.

The purpose of this work was to describe a novel approach to teaching anatomy-based safe and reproducible separation of the EDML from a corneoscleral button (donor preparation) and a stepwise approach for unfolding the graft in the AC (recipient preparation) in a well-equipped DMEK-Wetlab.

MATERIALS AND METHODS

Clinical Pearls

We ask all patients to have a large YAG laser iridotomy at the six o'clock position (not in the upper quadrant) some weeks before DMEK, to preclude bleeding or pigment dispersion in the event of intraoperative or the-day-before iridotomy.

We perform DMEK either sequentially in pseudophakic eyes (if the cataract is the leading pathology) or simultaneously with a phacoemulsification and posterior chamber intraocular lens implantation (so-called New Triple DMEK, if corneal edema is the leading pathology).

We consider the following donor details (Heinzelmann et al., 2014): (1) age of donor > 50 years because of easier unfolding in the AC; (2) diabetic donors avoided because of high risk of tearing during donor preparation (Greiner et al., 2014); (3) pseudophakic donors avoided—if possible—because of increased risk of radial tears near the incisions; (4) organ culture preferred for EDML stripping; (5) organ culture without osmotic pressure generator (e.g., Dextran) in a glass vessel to preserve "precut tissue" for up to two days after preparation only. Because of acceptable endothelial cell losses of 4.1% (first day), over 4.8% (fourth day), to 13.1% (tenth day) (Bayyoud et al., 2012), we improve our organization by preparing the donors stress-free in the operating theatre (OR) on Tuesday and Thursday afternoons. We store the EDML in organ culture medium (without Dextran) until the planned surgery on Thursday or Friday (Heinzelmann et al., 2017).

In real life, the pupil is maximally constricted for simple DMEK. For New Triple DMEK we avoid atropine drops and epinephrine in the AC during cataract surgery to allow the pupil to constrict as much as possible after implanting the posterior chamber IOL in the capsular bag.

In real life, after attaching the EDML, the AC is completely filled with air for 2–3 hr and the patient is asked to lie down on her/his back. Afterwards, the patient is taken back to the OR and a little fluid is instilled into the AC just to cover the iridotomy ($\sim$ 1/2 mm circular rim of peripheral fluid).

After surgery, patients are asked to lie down on their backs for four to five days. The first day we measure the intraocular pressure every 2 hr by applanation. After four to five days, we typically discharge the patient. To be on the safe side we see the patient again two weeks after surgery ("with suitcase and tooth brush"). At this time, we remove either the two corneoscleral single sutures or accommodate the patient for rebubbling if there is any (new) graft dehiscence. To reduce the risk of complications, the surgeon should indicate after DMEK which existing paracentesis should be used for rebubbling (we prefer an 80% fill of the AC with 20% SF_6 gas because of gas expansion)—ideally opposite the EDML detachment area to avoid iatrogenic folding of the graft.

The next outpatient follow-up examinations take place 6 weeks, 6 months, and 1 year postoperatively.

Our topical therapy consists of (1) hyperosmolar eye drops for 2 weeks, (2) antibiotics for 1 week, (3) prednisolone acetate hourly for one week to prevent cystoid macular edema (Heinzelmann et al., 2015; Hoerster et al., 2016) then five times per day to be tapered slowly over a period of 6 months, (4) lubricant as required (e.g., Hylogel 5× per day). After 6 weeks, temporary glasses can be prescribed.

Postoperative routine diagnostics include: (1) Best-spectacle-corrected visual acuity, (2) Slit lamp examination, (3) Applanation tonometry, (4) Pentacam tomography, (5) Endothelial cell count and, especially, (6) Anterior Segment OCT to assess the circular peripheral adhesion of the graft. Before discharge and during the outpatient control examinations, a macula OCT is performed to recognize and, if needed, to treat

Fig. 5. Operating table with all requisites for donor preparation. The same table is used for the Wetlab. [Color figure can be viewed at wileyonlinelibrary.com]

cystoid macular edema immediately (Heinzelmann et al., 2015; Hoerster et al., 2016).

The following patient education is indispensable: If (1) sight begins to worsen (mainly in the morning), (2) halos are seen around light sources or there is (3) segmental clouding, (4) the eye turns red or hurts, the patient should immediately see an ophthalmologist or come to our Department of Ophthalmology. Our motto: "Do not wait three days and hope . . ."

Additional Outcome Measures

All participants were asked to rate the two-day-long DMEK-Wetlab on a scale from 1 (excellent) to 6 (terrible). In addition, participants and tutors were asked to rate the potential ability of the participant to perform the donor and host DMEK procedure unaided after the Wetlab. For the assessment, we distinguish between participants of the Wetlab who are residents with no former experience in corneal surgery and those who are qualified ophthalmologists who have such experience.

Fig. 6. To prevent shifting during preparation of the EDML, the 15-mm corneoscleral buttons are positioned epithelium–down on a suction block, which is commonly used to prepare DSAEK tissue (Hanna trephination system; Moria SA, Antony, France). [Color figure can be viewed at wileyonlinelibrary.com]

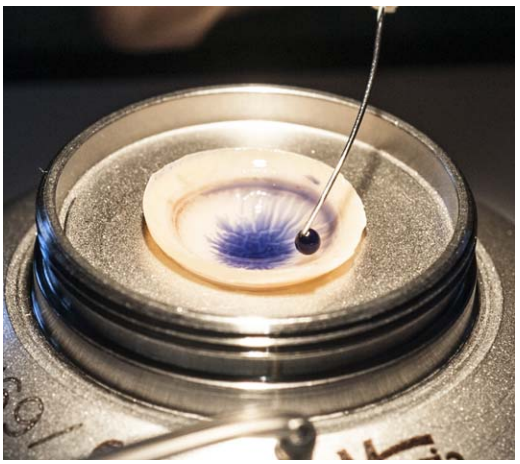

Fig. 7. To improve visualization, Blue Color Caps BCC (Croma GmbH, Leobendorf, Austria) staining is performed for about 60 sec. [Color figure can be viewed at wileyonlinelibrary.com]

RESULTS

Donor Preparation in the Wetlab

In the Wetlab we use the operating table that is prepared by nurses in the real world in our OR (Fig. 5). Only organ cultured buttons are used, either in medium with or without Dextran (Yoeruek et al., 2013). To prevent shifting during preparation of the EDML, the 15-mm corneoscleral buttons are positioned epithelium–down on a suction block (Fig. 6), which is commonly used to prepare DSAEK tissue (Hanna trephination system; Moria SA, Antony, France). After centering of the corneoscleral button, suction is turned on by a foot switch.

To improve visualization, Blue Color Caps BCC (Croma GmbH, Leobendorf, Austria) staining is performed for about 60 sec (Fig. 7). Afterwards, the BCC is replaced with organ culture medium. The endothelium must be kept moist at all times throughout the preparation. To facilitate orientation on the surface of the EDML, a very superficial 7.5-mm mark (using the 7.5-mm trephine of the Moria DSAEK trephination system) is introduced, with the same diameter as the future EDML transplant. Marking is achieved under visual control by gently sliding down the trephine within the guiding cylinder of the trephination system until it touches the surface of the endothelium (Fig. 8).

To access the margin of the EDML, peripheral lamellar incisions about 1.0 to 1.5 mm outside the 7.5-mm mark are cut hexagonally or octagonally with a razor blade (Fig. 9). This is necessary because the adhesion of DM to the underlying stroma is maximal at the very periphery at the limbus. For a right-handed surgeon, these incisions are best performed in clockwise order by rotating the suction block. At this point, the area of the incisions is again better visualized by applying BCC blue dye. Typically, DM is not only cut by the razor blade but also tears in a curvilinear way (by analogy with the capsulorhexis of the

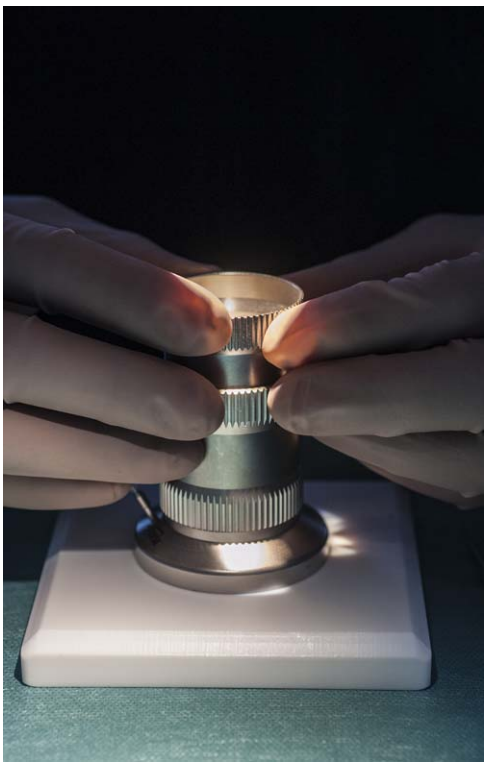

Fig. 8. Marking is achieved under visual control by gently sliding down the trephine within the guiding cylinder of the trephination system until it touches the surface of the endothelium. [Color figure can be viewed at wileyonlinelibrary.com]

lens; Fig. 10). Using a tiny nontoothed forceps, the DM is grasped radially with very little extension and its peripheral border (preferably a ruptured area) is lifted circularly by analogy with opening an envelope. Ideally, the central margin of the lifted DM should be 1 mm outside the 7.5-mm mark to allow the margin of the EDML to be lifted without reducing the number of endothelial cells in the future graft and to be able

Fig. 9. To access the margin of the EDML, peripheral lamellar incisions are made hexagonally or octagonally with a razor blade about 1–1.5 mm outside the 7.5-mm mark. [Color figure can be viewed at wileyonlinelibrary.com]

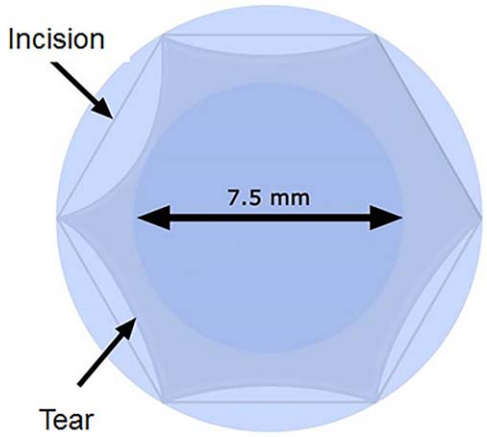

Fig. 10. Schematic: Typically, DM is not only cut by the razor blade but also ruptures in a curvilinear way (by analogy with the capsulorhexis of the lens). Using a tiny forceps, DM is grasped radially with very little extension and its peripheral border (preferably the ruptured area) is lifted circularly by analogy with opening an envelope. [Color figure can be viewed at wileyonlinelibrary.com]

to react appropriately if radial tears are induced in the EDML. Once a radial tear forms, the forceps has to be used for correction in a way similar to the manipulation of the anterior lens capsule during cataract surgery. Right-handed surgeons will best lift up the peripheral EDML in a counterclockwise direction by rotating the suction block.

Because endothelial cells outside the 7.5-mm zone will be removed anyway, grasping the margin outside the 7.5-mm zone with a forceps does not entail loss of endothelial cells on the graft and the EDML can safely be pulled toward the center. Both lateral edges of the slowly detaching EDML are watched closely so the pulling can be stopped immediately if a radial tear occurs. As planned, the pulling is stopped after about one third of the entire EDML is detached. Then the detached EDML is laid back in its original place. By rotating the suction block about 120°, the EDML in the second one-third can be separated from the stroma. During separation of the EDML in the final one-third, it is important to leave a small portion of it attached to the central stroma because otherwise it will start to float.

After the entire EDML is spread out in the correct anatomical position, lamellar trephination of it with the 7.5-mm trephine will result in a curvilinear edge, which is important for further manipulation of the tissue. The peripheral circular part of the EDML where endothelial cells were already injured during the initial detachment procedure is now discarded. Then, one half of the forceps is used to lift the partially incarcerated margins of the graft within the trephination groove to prevent tearing during the final detachment maneuver.

Most importantly, **orientation marks** are introduced to allow safe anterior–posterior orientation during the following steps of surgery, as described by

Fig. 11. Orientation marks are introduced to allow safe anterior–posterior orientation during the following steps of surgery as described by Bachmann et al. (2010). Three semicircular marks at the edge of the EDML are made with a 1-mm dermal trephine—two close to each other and one within a larger distance. [Color figure can be viewed at wileyonlinelibrary.com]

Bachmann et al. (2010; Fig. 11). Three semicircular marks at the edge of the EDML are made with a 1-mm dermal trephine—two close to each other and one at a greater distance (Fig. 12). The suction block is rotated so that the three marks are directed toward the surgeon. For complete stripping of the EDML, one forceps is used to lift its margin opposite the orientation marks. Because most of the EDML is already stripped from the stroma, complete separation is relatively easy and safe (Fig. 13). However, the forceps has to be guided along the concavity of the cornoscleral button without touching the endothelium. When the stripping is complete, the suction of the Moria system is released. A larger forceps is used to transfer the completely detached EDML to the complete corneoscleral button as a vehicle to a covered 6-well plate filled in part with organ culture medium without Dextran.

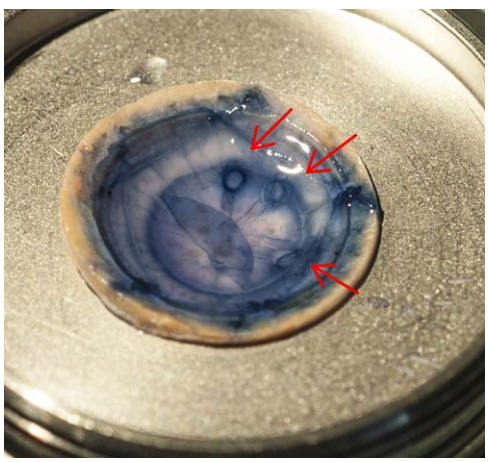

Fig. 12. Three semicircular marks at the edge of the EDML are made with a 1-mm dermal trephine—two close to each other and one within a larger distance. The contralateral part of the EDML is partly inverted for demonstration. [Color figure can be viewed at wileyonlinelibrary.com]

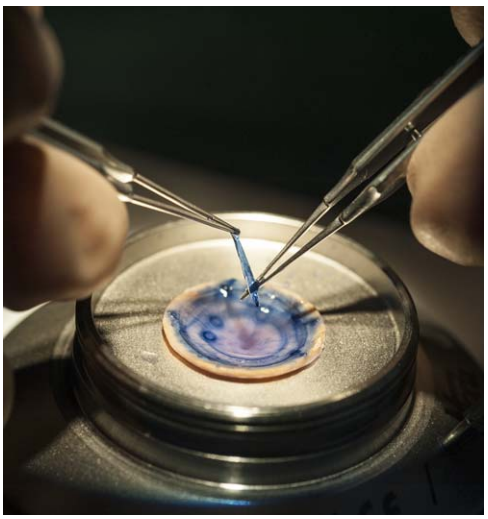

Fig. 13. The suction block is rotated so that the three marks are directed toward the surgeon. For complete stripping of the EDML, one forceps is used to lift the margin of the EDML opposite the orientation marks. Because most of the EDM is already stripped from the stroma, complete separation is relatively easy and safe. [Color figure can be viewed at wileyonlinelibrary.com]

The safety and speed with which the EDML graft is generated clearly improve with training. Although the preparation procedure takes up to one hour for beginners in the Wetlab, it can typically be done in 6–10 min by an experienced DMEK surgeon.

Recipient/Host Maneuvers in the Wetlab

Recipient preparation. A freshly enucleated pig eye stored in organ culture medium with Dextran is mounted in a special holder with four needles. An 8.0 mm ring-shaped mark is applied to the central corneal surface (Fig. 14) for a 7.5 mm graft. The donor diameter should always be smaller than the recipient diameter to prevent overlapping of donor and host DM, thus promoting sequential detachments and re-bubblings. A clear corneal phako incision (width 2.5 mm; Fig. 15A) and three small paracenteses (width 1.8 mm) at a distance of 120° are made at the limbus (Fig. 15B). The length of the phako incision (length about 1.5 mm) should ensure that the distance between the inner lip of the tunnel and the contralateral chamber angle allows enough space to deliver the graft and various maneuvers with a cannula via the tunnel. Through one of the paracenteses, an AC maintainer attached to a vitrectomy unit is placed at the limbus to enable a centered descemetorhexis to be achieved under air (Fig. 16). For this, an inverted hook (Price Endothelial Keratoplasty Hook; Moria SA) is introduced via a paracentesis opposite the AC maintainer (Fig. 17). Removal of the DM is the same as during DSAEK surgery. Unfortunately, this

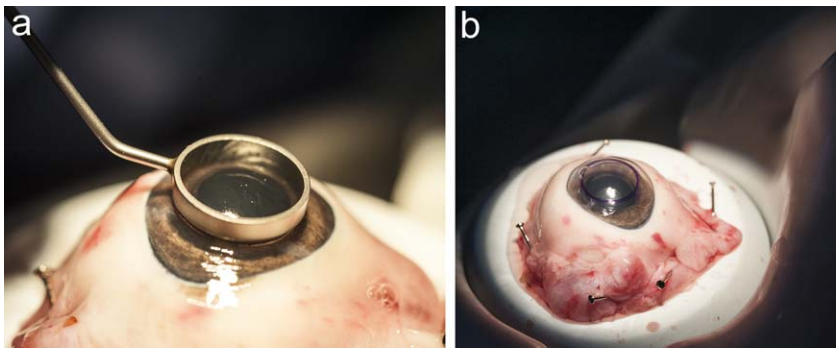

Fig. 14. A freshly enucleated pig eye stored in organ culture medium with Dextran is mounted on a special holder with four needles. An 8.0 mm ring-shaped mark is applied to the central corneal surface for a 7.5 mm graft. [Color figure can be viewed at wileyonlinelibrary.com]

step cannot be simulated in a young pig eye because DM cannot be removed without major destruction owing to its firm adhesion to the overlying stroma. Afterwards, the paracentesis that has hosted the AC maintainer is closed with a 10–0-Nylon single suture and the AC is left filled with air.

Preparation of the EDML injection system. The EDML roll prepared the day before by the same participant and preserved in a covered 6-well plate is transferred to a glass container (12×1 cm^2) half filled with organ culture medium without Dextran. The dark blue dye DORC Membraneblue Dual (Dorc, VN Zuidland, The Netherlands) is injected into the lumen of the roll via a tiny cannula. A Single Use DMEK Glass Cartridge produced by Geuder (Heidelberg, Germany) is completely submerged in the medium and is connected to a tube and a 5 ml syringe (from the Geuder set). Using forceps, the rolled membrane can easily be manipulated into the large opening of the glass cartridge groove. To facilitate this procedure, the assisting nurse pulls the plunger of the syringe gently (Fig. 18). Then another three ml syringe is connected to the large opening of the glass cartridge and the tube is removed from its tip. The roll is moved to the tip of the cartridge by gently pushing the three ml plunger (Fig. 19). The dark blue dye DORC Membraneblue Dual (Dorc, VN Zuidland, The Netherlands) is introduced via a tiny cannula into the tip of the glass cartridge until the entire roll is covered with it (Fig. 20). This step is clinically of utmost importance to ensure that all three marks at the border of the EDML are easily appreciated later in the AC. Then we return to the pig eye and wait 30 sec to be sure that the dye has been taken up by the EDML.

Insertion of EDML. To prepare for insertion of the EDML roll, the air is completely removed and the AC is filled with balanced salt solution (BSS). The tip of the glass cartridge is inserted "face down" toward the mid-periphery of the AC to avoid hiding the graft behind the iris. By pushing the plunger, the EDML roll including the blue fluid in the glass cartridge is "shot" into the AC (Fig. 21). Then the incision is closed with a 10–0-Nylon single suture on a toned globe without disturbing the corneal topography too much (Fig. 22).

Orientation of EDML in the AC. Using a small cannula and BSS the blue dye is washed out of the AC. This typically tends to open the EDML roll partly, allowing the surgeon to appreciate the margin of the graft. It is most welcome if the EDML forms a triangular "Napoleon's hat." Observation of the marks on the edge of the membrane together with observation of the edge of the roll (which curls upward against the back of the cornea) allows the correct orientation to be confirmed. At this stage, the graft can be unfolded

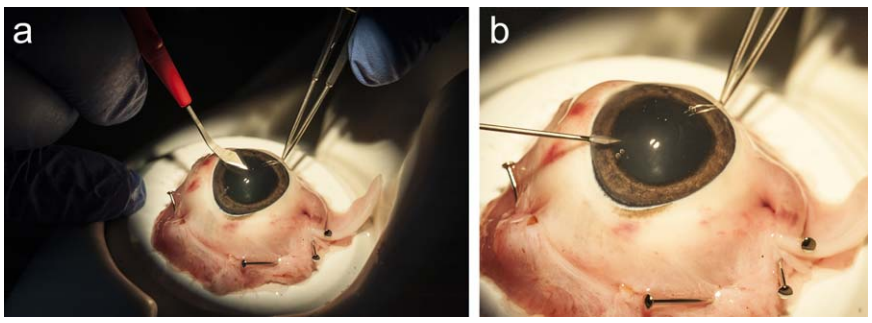

Fig. 15. **A**: A clear corneal phako incision (width 2.5 mm) and **B**: three small paracenteses (width 1.8 mm) at a distance of 120° are made at the limbus. [Color figure can be viewed at wileyonlinelibrary.com]

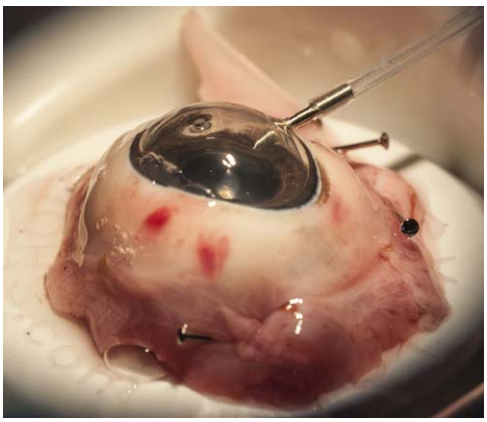

Fig. 16. Via one of the paracenteses, an AC maintainer attached to a vitrectomy unit is placed at the limbus. [Color figure can be viewed at wileyonlinelibrary.com]

either by tapping on the host cornea after having pressed on the bottom of the phakotunnel to flatten the AC, or by inserting a small air bubble into the inner lumen of the EDML roll. By tapping appropriately on the cornea, the air bubble is used to unfold the margins of the EDML sequentially. If the membrane cannot be centered by indirect means, it sometimes seems advisable to use a small spatula via the tunnel to center it gently by direct means. If the AC is too deep, the membrane can be spread out on the surface of the iris by increasing the air bubble and filling the AC completely but without too much pressure. In any case, the small or large air bubble has to be sucked out carefully. The cannula has to be removed without disturbing the EDML. Then, a cannula attached to a completely air-filled syringe has to be forwarded UNDER the EDML toward the center of the pupil. During this stage, the surgeon's thumb has to be away from the plunger to avoid a "disaster." By injecting air, the EDML is typically attached to the back of the host cornea like a jellyfish. Observation of the orientation marks allows the correct orientation of the graft to be

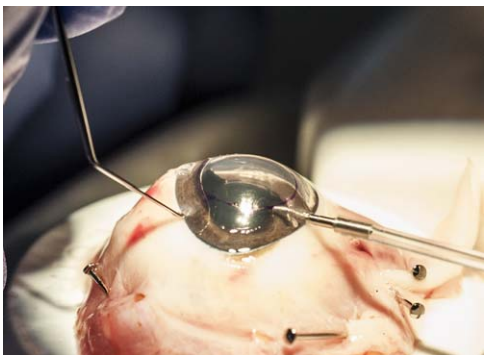

Fig. 17. For this, an inverted hook (Price Endothelial Keratoplasty Hook; Moria SA) is introduced via a paracentesis opposite the AC maintainer. [Color figure can be viewed at wileyonlinelibrary.com]

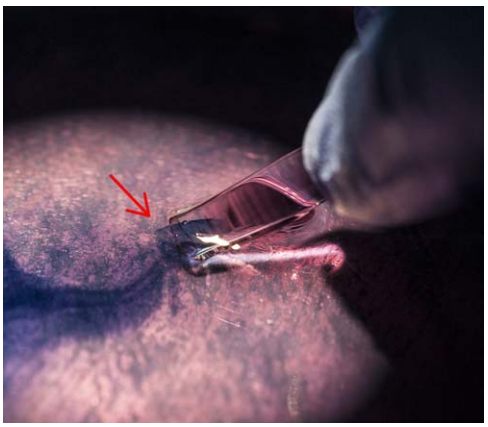

Fig. 18. Using a forceps, the rolled membrane can easily be manipulated into the large opening of the glass cartridge grove. To facilitate this procedure, the assisting nurse gently pulls the plunger of the syringe. [Color figure can be viewed at wileyonlinelibrary.com]

confirmed (Fig. 23). It is very important to ensure a central location before inserting the air bubble under the EDML, because in contrast to DSAEK, the EDML graft can seldom be moved much once it is attached to the host stroma. Some shifting can sometimes be achieved by vigorously tapping a squint hook from the limbus to the center in the hemimeridian of the decentration.

Assessment of participants. Twenty-two candidates in the **First Homburg Cornea Curriculum *HCC 2015*** who practiced both steps on three human donor corneas and three pig eyes under the guidance of an experienced corneal surgeon assessed the procedure as follows: (1) Overall, the grade of the Wetlab was 1.4 (Median 1, range 1–2-on a scale from 1 (excellent) to 6 (terrible). (2) Most participants and tutors stated that the Wetlab is most effective for colleagues who have some previous experience with corneal microsurgery. The Wetlab described here makes no sense for residents who have never performed intraocular surgery. Therefore, it is no longer available for residents but only for qualified ophthalmologists with some previous experience of corneal surgery.

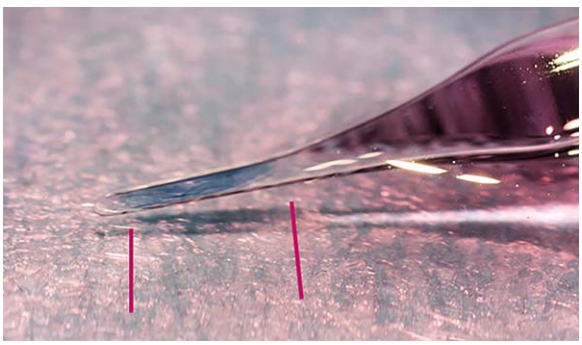

Fig. 19. The roll is moved to the tip of the cartridge by gently pushing the three ml plunger. [Color figure can be viewed at wileyonlinelibrary.com]

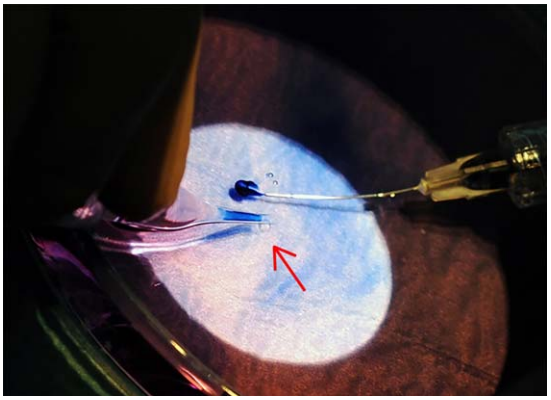

Fig. 20. The dark blue dye DORC Membraneblue Dual (Dorc, VN Zuidland, The Netherlands) is introduced via a tiny cannula into the tip of the glass cartridge until it covers the entire roll. This step is clinically of utmost importance to ensure that all three marks at the border of the EDML are easily appreciated later in the AC. [Color figure can be viewed at wileyonlinelibrary.com]

DISCUSSION

DMEK in Clinical Reality

DMEK has become very widespread in Germany over the last five years, with a proportion of >50% of all keratoplasties performed (Maier et al., 2013). Its advent allows the host's diseased endothelium to be replaced practically without alteration of the posterior surface of the cornea (Melles, 2006; Neieuwendaal et al., 2006; Price and Price, 2006). Our own experience during the learning process showed that each step in donor preparation and host maneuvers during DMEK can potentially lead to disaster. Thus, the goal for the incipient DMEK surgeon must be to standardize the procedure as much as possible beforehand, leaving only the unfolding of the 20 to 30 μm thick EDML

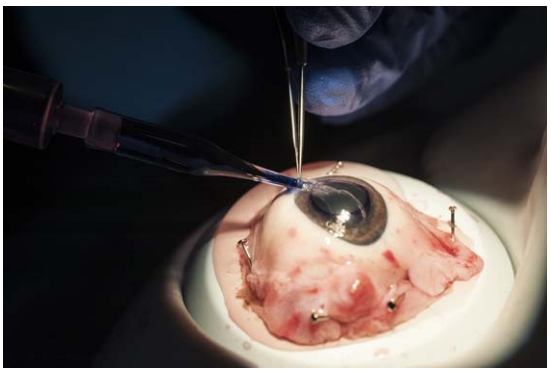

Fig. 21. To prepare for insertion of the EDML roll, the air is completely removed and the AC is filled with BSS. The tip of the glass cartridge is inserted "face down" into the mid-periphery to the AC to avoid hiding the graft behind the iris. By pushing the plunger, the roll including the blue fluid in the glass cartridge is "shot" into the AC. [Color figure can be viewed at wileyonlinelibrary.com]

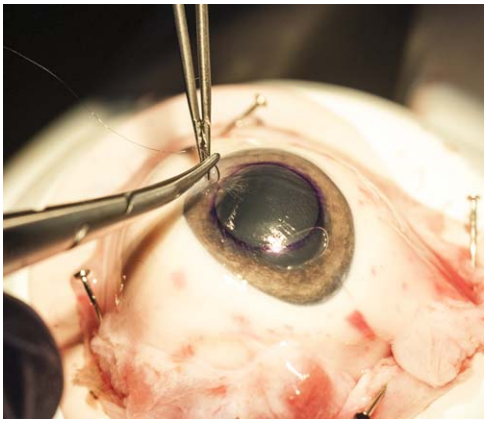

Fig. 22. Then, the incision is closed with a 10–0-Nylon single suture on a toned globe without disturbing the corneal topography too much. [Color figure can be viewed at wileyonlinelibrary.com]

in the AC as potentially "surprising" (Kruse et al., 2011). However, even this can be "standardized" by a well-established escalation plan as soon as the incision is closed with a 10–0 Nylon stitch to prevent the EDML from slipping out of the AC.

Previously, incipient DMEK surgeons were supposed to watch many videos and PowerPoint slides to prepare themselves for their first case or to watch a master in the operating theater. In wetlabs, typically only pig eyes have been used. Since DM cannot easily be stripped in young pig eyes, no effective learning for clinical application could be achieved in respect of DMEK.

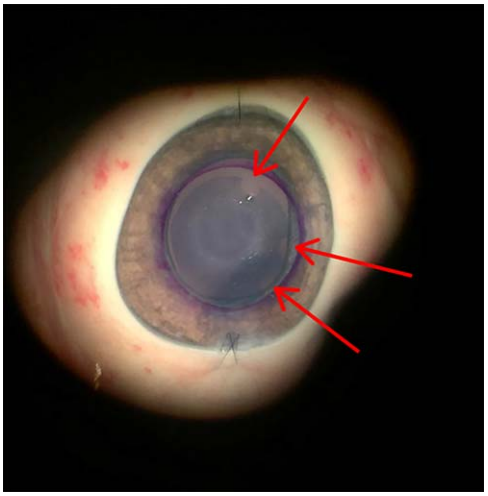

Fig. 23. The cannula attached to a completely air-filled syringe has to be forwarded UNDER the EDML toward the center of the pupil. During this stage, the surgeon's thumb has to be away from the plunger to avoid a disaster. By injecting air, the EDML is typically attached to the back of the host cornea like a jellyfish. Observation of the orientation marks (arrows) allows their correct orientation to be confirmed. [Color figure can be viewed at wileyonlinelibrary.com]

We therefore collected all corneas not suitable for transplantation in our LIONS EYE BANK Saar-Lor-Lux, Trier/Westpfalz over one year to offer each participant in the Wetlab the chance to prepare at least three donors under 1:1 guidance by an experienced corneal surgeon (Li et al., 2008; Heinzelmann et al., 2014).

Major remaining uncertainties for the incipient DMEK surgeon include (1) the quality of the donor and the (2) unfolding (and attachment) of the EDML roll in the AC. Concerning problem (1) we use only donors older than 50 years and prefer eyes without previous cataract surgery (because of the lamellar incisions that can cause radial tears during donor preparation), and we do not use corneas from diabetic donors because the rate of unexpected (paracentral) tearing is significantly higher according to the literature (Greiner et al., 2014). If small radial tears occur during donor preparations, we grasp the edge with forceps and complete the radial tear to a semicircular concave edge (as in capsulorhexis for cataract surgery), and we mark this critical area at the scleral rim. If the size of the tear is within the limit of the mark, it is covered by trephination with the 1-mm trephine so it is incorporated into the pattern of the margin of the EDML graft, which always contains three orientation marks (Bachmann et al., 2010, Kruse et al., 2011).

At present, it is unclear how endothelial cells are best preserved during and after preparation of the EDML graft. Many factors affect the viability of the endothelium such as storage conditions before surgery, the media used during preparation, and the time point of preparation relative to the time of surgery. In contrast to previous reports (Kruse et al., 2011; Heinzelmann et al., 2017), we opted to prepare the EDML not immediately before surgery but one or two days before, and to preserve the grafts in organ culture medium without Dextran. Thus, potential organizational problems in the event of destruction of donor tissue can easily be resolved. Of course, this problem does not exist in principle if an adjunct eye bank always has a spare cornea available in case of adverse events in the OR.

To strip off of the diseased host DM, an AC maintainer attached to a vitrectomy unit is placed at the limbus via one of the paracenteses. Thus, the AC is preserved throughout the entire descemetorhexis. This helps enormously in real patients with vis-a-tergo (= "positive vitreous pressure"). In the Wetlab, we can only simulate a centered descemetorhexis under air with the appropriate instrument. Unfortunately, this step cannot be performed in a young pig eye because DM cannot be removed without major destruction owing to its firm adhesion to the overlying stroma.

Another fundamental step in determining the success of a DMEK procedure is unfolding the EDML graft in the AC. With respect to manipulation of the graft in the AC, DMEK surgery still differs from other eye surgeries such as PKP or even DSAEK, which are standardized procedures. In DMEK surgery, the way the procedure is conducted is largely determined by the initial position of the graft in the AC. Furthermore, the dimensions of the AC are critical. Thus, preoperative selection is more important than in other kinds of ophthalmic surgery. We feel that a graft diameter of 7.5 mm is rarely disadvantageous (one size fits "all").

Concerning the problem of unfolding (and attachment) of the EDML roll in the AC, we have three principal approaches that should help to overcome all surprises. If the AC becomes flat easily, as we wish, we can often spread out the EDML without air just by tapping on different parts of the host cornea mono- or bi-manually. If this is not possible, we introduce a small air bubble into the center of the EDML. If the AC stays too deep throughout the procedure, we use the big bubble to press the EDML down to the iris completely. After carefully sucking out this air (lumen of the cannula toward the host stroma), the cornea typically collapses and the EDML stays on top of the iris.

If the roll is very tight (from a young donor), we try to open it either by multiple fluid shots via different paracenteses or we use a LASIK instrument to wash the interface with holes on both sides of the cannula to help unroll the EDML in the AC. In addition, we work closely with the anesthesiologist to achieve a mild to moderate vis-a-tergo for helpful flattening of the AC during unfolding of the EDML. Vitrectomized eyes with constantly deep AC need a modified approach and are not part of this DMEK Wetlab.

Future

The following questions regarding DMEK need to be clarified scientifically:

1. What is the most reliable donor preparation procedure?
2. How much indirect (and direct?) intraoperative manipulation can the endothelium tolerate?
3. Do glass and plastic (storage and shooter) affect the endothelial cell count and graft detachment rate?
4. Are immune reactions really so rare or are they just overlooked in some instances (Anshu et al., 2012)?
5. How long and in which medium and in which vessel can "precut tissue" be stored without major reduction of endothelial quality (Heinzelmann et al., 2017)?
6. What will be the repeat surgery rate 10 or 20 years from now (Price et al., 2009; Baydoun et al., 2012)?

CONCLUSIONS

In Germany today, DMEK is the method of choice in cases of isolated endothelial defects without stromal scars. The increase in the number of keratoplasties in Germany over the last five years has most likely resulted from increases of indication (Fuchs dystrophy with visual acuity 20/25 or even 20/20) and of repeat keratoplasty because of early decompensated grafts after DMEK in beginners' hands, and needs to be further observed and scientifically assessed.

The technique taught in the Wetlab must be easy to follow, easy to repeat, reliable, free of mystery or hocus pocus, and contain as little "individual artwork" as possible. Our novel approach to anatomy-based simulated donor preparation and graft implantation for DMEK seems to meet the needs of incipient DMEK surgeons with some previous experience in corneal surgery and will help to reduce the rate of donor destruction and implantation failure during DMEK in the future.

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